PLASMA AND ORGAN BIOCHEMISTRY OF *Clarias gariepinus* EXPOSED TO MONOAROMATIC, TOLUENE

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ABSTRACT

The effect of toluene on enzymes; Aspatate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in the plasma, organs (gills, kidney, liver) and muscle of Clarias gariepinus was assessed. The activity of AST in the gill was variable among the treated group and between the treated groups and the control with increase in all the concentrations (129.4 – 976%) above the value of the control (42.50 ± 28.72 IU/L). Activity of ALT in the gill was gradually raised from 50 ppm to a peak (187.50%) at 125 ppm. Although the ALP had the highest activity in the gill in comparison to other enzymes it was variable with inhibitions. Toluene caused excitation at 75 and 125 ppm were more than thrice and twice respectively that at the control in the kidney. It caused increase in the activity of ALT with more than 6x (25 ppm), 9x (75 ppm) and 15x (125 ppm) that of the control. However, the activity of ALP in the kidney was generally unaffected by the exposure at the other concentrations. There was general excitation of AST activity in the liver with higher levels of excitation, 25 – 40% at 25, 75 and 125 ppm. A raise in ALT activity was recorded in all the exposure. A concentration-dependent increase was observed in activity of ALP in the liver 299, 479 and 596% above the control value, 46.00 ± 34.84 IU/L. There was both excitation inhibition of AST activity in the muscle tissue of the fish. There was a sharp decline of 69 and 55% at 50 and 100 ppm in ALT activity and enhanced activity of 321, 179 and 227% at 25, 75 and 125 ppm, respectively above the control value. ALP activity was excited in the muscle in all the test concentrations (maximum, 90% at 25 ppm). There was a sharp decline in the activity of AST in the plasma with the least value at 75ppm, 64% lower than the control value. All concentrations toluene elicited the activity of ALT in the plasma.. The increase were 3.5x, 2.5x, 4.5x, 2.25x and 5x at the exposure concentrations (25, 50, 75, 100 and 125 ppm) respectively, relative to the control values. The relative activity of AST in the organs generally followed the pattern: muscle > liver > gill > kidney> plasma and that of ALT activity, muscle > kidney > liver > gill > plasma and ALP was kidney > muscle > liver > gill > plasma.

Keywords: Toluene, *Clarias gariepinus,* Aspartate transaminase, Alanine transaminase, Alkaline phosphatase

INTRODUCTION

Over the years there is constant contamination of the environment, particularly in the Niger Delta, Nigeria by petroleum hydrocarbons from numerous outlets by petroleum and allied products from and exploration and exploitation (Benson and Essien, 2009). This comes from anthropogenic sources which include offshore oil production, transportation, atmospheric and aerial depositions, direct dumping and accidental discharge among others (Abu-Hilal and Khordagui, 1994; NRC, 2000) which are capable of causing chronic effects in the water settlements. The yearly entry of crude oil into the aquatic environment is in the region of between 6 – 10 million barrels (Thorhang, 1992). Control of this problem in the aquatic environment is very difficult die to the large number of input sources, their geographic dispersions and the rate at which the products mixes with water and the resultant effect of their components on aquatic organisms (Patin, 1991; Dambo,1992).

Toluene is a low molecular weight aromatic monocyclic hydrocarbon. It is a major component of water soluble fractions of crude and refined petroleum products. It is released into the atmosphere principally from volatilization of petroleum fuels and toluene-based solvents and thinners, and from motor vehicle exhaust. Considerable emissions are from its discharge into waterways or spills on land, transport and disposal of fuels and oils; from its production from petroleum and coal, as a by product from styrene production and from its use as a chemical intermediate (EPA, 1994). The source further indicated that the amount of toluene released to land and water was totaled over 4 million Ibs from 1987 – 1993. Toluene released into water can take few days to several weeks to remove depending on the environmental condition. Like other volatile hydrocarbons, toluene is reported toxic to many aquatic life forms including fish with varying 96hr median lethal concentrations that were species, size and duration-dependent (Blacks *et al.,* 1982; Rice and Thomas, 1989; Kennedy *et al.,* 2006)

Most of the studies conducted on the physiological effects of oil pollution in aquatic fauna in Nigerian waters dealt with whole crude oil (Dambo, 1992; Ovuru and Mgbere, 2000), refined products (Ayalogu *et al.,* 2001; Gabriel *et al*., 2009), the water soluble fractions (Osuji and Mbata, 2004), but the effects of the low boiling points components (monoaromatic hydrocarbons) which are considered the most toxic constituents have received little of no attention. Petroleum oils and the aromatics have been shown to impact negatively on the physiology of important fish species (Dange and Masureka, 1981; Abu-Hilal and Khordagui, 1994; NRC, 2000). In the Niger Delta region which has heavy concentration of oil installations and oil-related activities and aquaculture production where the catfishes especially *Clarias gariepinus* is intensively cultured, minimal or no attention has been given to the possible effects of the monoaromatics on the physiology of the fish species despite the rampant spills recorded in the region. Therefore, this study was carried out to assess the possible effects of toluene on the enzyme activities in the blood (plasma) and organs of *C. gariepinus* under laboratory conditions.

MATERIALS AND METHODS

The experimental fish, *C. gariepinus* (mean total length, 166.01 ± 15.0 cm; mean weight, 30.15 ± 2.27 g) was procured from a private farm, Ellah Lakes, Obrikom, Rivers State, Nigeria and transported in unaerated aquaria to the Chemistry Laboratory, Rivers State University Science and Technology, Port Harcourt, Rivers State, Nigeria. Catfishes were acclimated individually in 10/ water to laboratory condition in plastic aquaria for seven days. The fish were fed once daily on a 30% crude protein diet at one percent biomass. The aquaria were carefully washed and cleansed of uneaten food and feacal matters by siphoning after which the water also renewed daily.

At the end of the acclimation period, the fish was exposed individually in quadruplicates to graded levels of toluene (0.00, 25.00, 50.00,

75.00, 100.00 and 125 ppm), respectively for 21 days in a daily renewal bioassay. The fish were fed as in the acclimation period and toxicant was renewed daily. At the end of the experimental period, blood samples were collected from the kidney with a 21 G size needle and a 5 ml syringe into heparinised bottles for enzyme analysis. After this the fish were anaesthetized, dissected to expose the viscera and the organs (gill, kidney, liver) and muscle were excised from the fish. Samples (0.5g) of the organs were macerated and homogenised in a mortar with 5 ml of physiological saline for enzymatic studies. Both the blood and the organs samples were centrifuged at 3000 rpm for 10 minutes and the supernatant pipetted into plain bottles analysis of enzymatic activities.

Aspartate transaminase, AST (EC 2.6.1.1) and alanine transaminase, ALT (EC 2.6.1.2) activities were assayed using the procedures of Reitman and Frankel (1957). Alkaline phosphatase, ALP (EC 3.1.3.1) activity was assayed using the methods of Babson *et al*. (1996). The data obtained were subjected to a one way analysis of variance (ANOVA). Where differences existed in the parameters, Duncan's multiple range test (DMRT) was used to separate the means (Zar, 1984).

RESULTS

The activity of AST in the gill was variable among the treated group and between the treated groups and the control. There was increase of activity in all the concentrations (129.4 – 976%) above the value of the control, 42.50 ± 28.72 iu/l. The activity of ALT in the gill was gradually raised from 50 ppm to a peak (187.50%) at 125 ppm. ALP had the highest activity in the gill in comparison to other enzymes. However, the activity was not concentration dependent. The activity levels were variable with inhibition at 50, 100 and 125 ppm and excitation of 17.84 and 19.96% at 25 and 75 ppm, respectively (Table 1). In the kidney, exposure to toluene caused variable levels of excitation of AST activity in all the exposure concentrations above the control value. The excitation at 75 and 125 ppm were more than thrice and twice respectively that at the control. Exposure to toluene caused increased in the activity of ALT with more than 6 times at 25 ppm, 9 times at 75 ppm and 15 times at 125 ppm when compared with the control. However, the activity of ALP in the kidney was generally unaffected by exposure to the other concentrations (Table 2). There was general excitation of AST activity in the liver with higher levels of excitation, 25-40% at 25, 75 and 125ppm (Table 3). ALT activity was recorded in all the exposure concentrations with higher activity 369, 372 and 751% above the control value at 25, 75 and 125 ppm, respectively. A concentration-dependent increase was also observed in activity of ALP in the liver 299, 479 and 596% above the control value, 46.00 ± 34.84 IU/L (Table 3). There was both excitation inhibition of AST activity in the muscle tissue of the fish (Table 3). Excitation of 22 and 17% above control values were recorded at 25 and 75 ppm, respectively; while inhibition values of 11 and 4% were recorded at 50 and 100 and 125 ppm, respectively. There was a sharp decline of 69 and 55% at 50 and 100 ppm in ALT activity and enhanced activity of 321, 179 and 227% at 25, 75 and 125 ppm, respectively above the control value. ALP activity was excited in the muscle in all the test concentrations (maximum, 90% at 25ppm). However, the excitation decreased with increase the test concentration. The activities ranged from 4 – 90% in the reverse order (Table 4). There was a sharp decline in the activity of AST in the plasma with the least value at 75 ppm, 64% lower than the control value. All concentrations toluene elicited the activity of ALT in the plasma. The increase were 3.5x, 2.5x, 4.5x, 2.25x and 5x at the exposure concentrations (25, 50, 75, 100 and 125 ppm), respectively relative to the control values (Table 5). The relative activity of AST in the organs generally followed the pattern: muscle > liver > gill > kidney> plasma and that of ALT activity, muscle > kidney > liver > gill > plasma and ALP was kidney > muscle > liver > gill > plasma (Figures 1 – 3).

Table 1: Activities of enzymes (AST, ALT and ALP) the gill tissue of *Clarias gariepinus* after exposure to toluene for 21 day

Conc.	AST (iu/l)	% difference from control	ALT (iu/l)	% difference from control	ALP (iu/l)	% difference from control
0	42.50 ± 28.72b	100	10.00 ± 0.00a	100	511.25 ± 34ab	100
25	152.50 ± 82.61b	358.6	15.00 ± 7.07a	150	602.50 ± 23.27a	118
50	97.50 ± 23.63b	229.4	11.25 ± 2.5a	113	450.63 ± 81.56ab	88
75	575.00 ± 119.48a	130.3	18.33 ± 7.64a	183	613.33 ± 10.41a	120
100	61.25 ± 28.39b	144.03	18.75 ± 14.36a	188	346.67 ± 237.16b	68
125	500.00 ± 177.55a	1176	28.33 ± 32.75a	283	498.07 ± 124.25ab	97

Means in the same column with the same alphabets are not significantly different at p<0.05 (DMRT)

Table 2: Activities of enzymes (AST, ALT and ALP) in the kidney tissue of *Clarias gariepinus* after exposure to toluene for 21 days

Conc.	AST (iu/l)	% difference from control	ALT (iu/l)	% difference from control	ALP (iu/l)	% difference from control
0	131.25 ± 62.25b	100	15.00 ± 7.07c	100	542.5 ± 20.26a	100
25	243.75 ± 94.46b	185	97.50 ± 38.62b	650	498.25 ± 44.11a	91.8
50	230.00 ± 199.54b	175	17.50 ± 5.00c	117	548.75 ± 19.31a	101
75	440.00 ± 103.32a	335	146.67 ± 20.21b	998	605.00 ± 22.91a	112
100	156.00 ± 18.43b	119	25.00 ± 8.16c	167	556.25 ± 4.79a	103
125	325.00 ± 134.26ab	248	203.33 ± 75.12a	1556	486.67 ± 230.99a	90

Means in the same column with the same alphabets are not significantly different at p<0.05 (DMRT)

Table 3: Activities of enzymes (AST, ALT and ALP) in the liver tissue of *Clarias gariepinus* after exposure to toluene for 21 days

Conc.	AST (iu/l)	% difference from control	ALT (iu/l)	% difference from control	ALP (iu/l)	% difference from control
0	462.50 ± 317.29a	100	16.25 ± 6.29c	100	46.00 ± 34.84c	100
25	647.50 ± 44.44a	140	76.25 ± 34.73b	469	114.25 ± 36.65bc	248
50	468.75 ± 202.75a	101	17.50 ± 5.00c	108	156.13 ± 115.65bc	339
75	601.07 ± 27.54a	130	76.67 ± 20.21b	472	183.33 ± 58.53ac	399
100	501.25 ± 247.94a	108	23.75 ± 15.48c	146	266.13 ± 69.82ab	579
125	576.67 ± 68.98a	125	138.33 ± 45.09a	851	320.33 ± 185.91a	696

Means in the same column with the same alphabets are not significantly different at p<0.05 (DMRT)

Table 4: Activities of enzymes (AST, ALT and ALP) in the muscle tissue of *Clarias gariepinus* after exposure to toluene for 21 days

Conc.	AST (iu/l)	% difference from control	ALT (iu/l)	% difference from control	ALP (iu/l)	% difference from control
0	586.25 ± 132.5a	100	52.50 ± 29.01b	100	22.50 ± 9.77b	100
25	655.00 ± 150.11a	122	221.25 ± 84.30a	421	422.75 ± 12.92a	190
50	520.00 ± 0.00a	89	17.50 ± 5.00b	33	29.00 ± 16.28ab	129
75	688.33 ± 14.43a	117	146.67 ± 82.51b	279	28.50 ± 1.80ab	127
100	520.00 ± 0.00a	89	23.75 ± 15.48a	45	25.50 ± 6.48b	113
125	561.67 ± 165.86	96	171.67 ± 55.08	327	23.33 ± 4.86b	104

Means in the same column with the same alphabets are not significantly different at p<0.05 (DMRT)

Table 5: Activities of enzymes in the plasma tissue of *Clarias gariepinus* after exposure to toluene for 21 days

Conc.	AST (iu/l)	% difference from control	ALT (iu/l)	% difference from control	ALP (iu/l)	% difference from control
0	56.00 ± 69.19	100	2.00 ± 1.41	100	15.30 ± 4.41	100
25	26.75 ± 10.75	48	6.50 ± 3.32	325	13.85 ± 4.16	91
50	50.75 ± 2.31	91	4.50 ± 3.70	225	19.00 ± 14.54	124
75	20.33 ± 34.00	36	9.00 ± 6.08	450	18.33 ± 18.33	120
100	53.00 ± 12.53	95	4.25 ± 1.50	213	25.00 ± 8.39	163
125	28.00 ± 3.54	50	10.00 ± 6.25	500	18.33 ± 12.06	120

Means in the same column with the same alphabets are not significantly different at p<0.05 (DMRT)

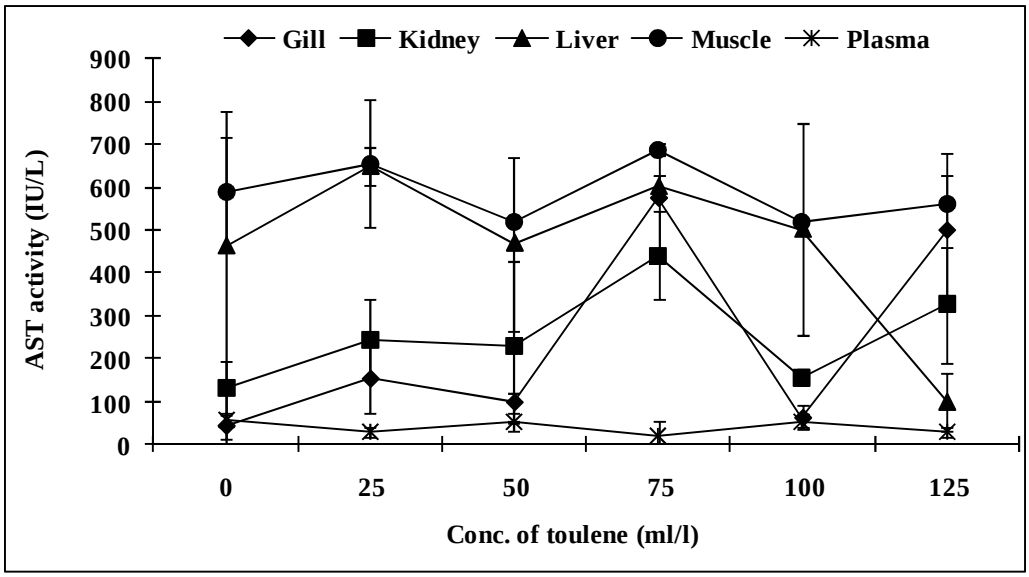

Figure 1: Relative activity of AST in selected tissues of *C. gariepinus* exposed to toluene for 21 days

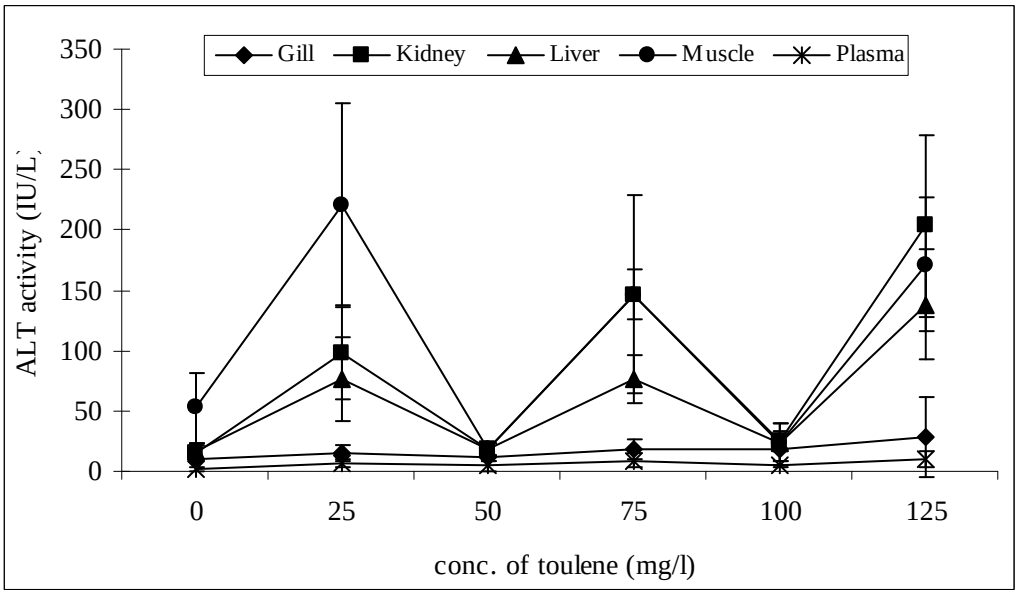

Figure 2: Relative activity of ALT in selected tissues of *C. gariepinus* exposed to toluene for 21 days

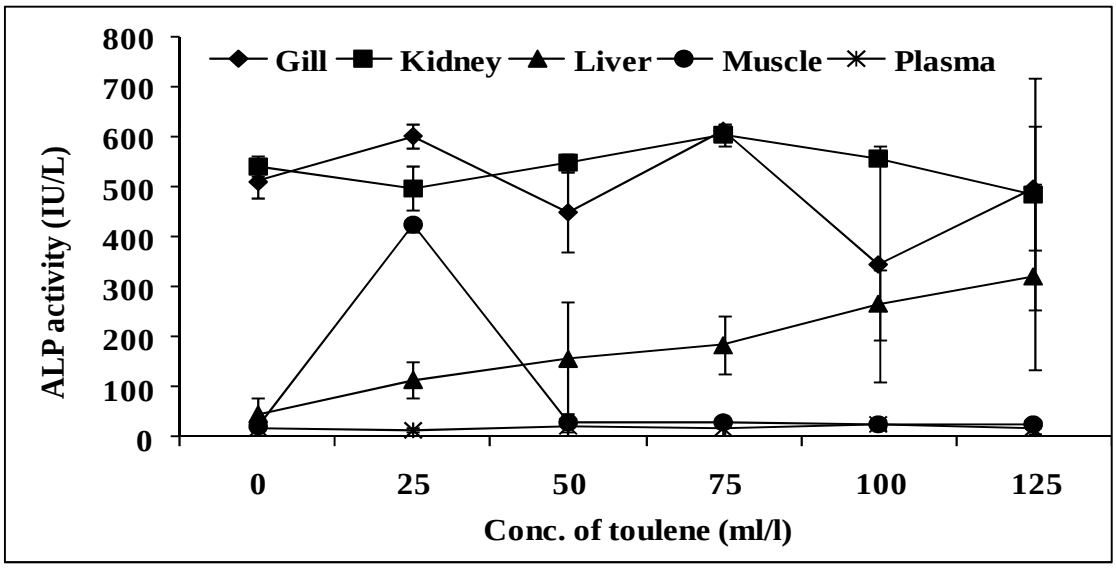

Figure 3: Relative activity of ALP in selected tissues of *C. gariepinus* exposed to toluene for 21 days

DISCUSSION

Disruption of the integrity of the cells of organisms is often measured by the changes in biochemistry and physiology of the organism (de la Torre *et al.,* 2000). Biomarkers such as enzymes (AST, ALT and ALP) are some of the most sensitive markers employed in the diagnosis of health condition of organisms under contaminant exposure because they are cytoplasmic in location and are released into circulation after cell damage (Pari and Murugavel, 2004). Treatment of the fish *C. gariepinus* with toluene generally increased the activities of the enzymes AST, ALT and ALP in all the organs (liver, gill, kidney and muscle) above the control value but decreased activities of these enzymes were recorded in the plasma of the fish. However, changes in activities of these enzymes have been observed in other studies (Ugwu *et al.,* 2008; Ozmen *et al.,* 2008).

The elevation of the transaminases is an indication of stress, a consequence of toluene

exposure reported for fish exposed to other environmental contaminants (Tiwari and Singh, 2004). The aminotransferases respond to stress or changes in physiological condition which in most cases leads to elevation of their activities (Natarajan, 1985). To overcome or combat stress, fishes need more energy to so that the demand for carbohydrate and its precursors could be maintained, therefore there is increasing step of the activities of the transaminases to cope with such energy requirement (Tiwari and Singh, 2004) and maintenance of the glycolytic pathway. Increase in the activities of these enzymes in the organs could be due to internal synthesis of the enzyme molecules, a pathway representing anaerobic tendencies of the tissues toward toluene toxicity or a counter adaptive measure to the assault of the toluene (Yakubu *et al*., 2001) leading to higher activities than in the control. Furthermore, the increased level of the transaminases after exposure to toluene may be due to changes in enzyme activity resulting from disturbance in the Kreb's cycle (Salah El-Deen and Rogers, 1993). However, the decreased activity in the plasma of all the enzymes (AST, ALT and ALP) suggests that the structural integrity of the cells membrane of the various organs were preserved and protected (Pari and Amali, 2005). The aminotransferases occupy a central position in the amino acid metabolism to form new amino acids during the degradation of amino acid and are also involved in the biochemical regulation of intracellular amino acid pool (Yakubu *et al*., 2005).

ALP is an important biomarker enzyme due to its involvement in adaptive cellular response to environmental toxicants (Lohner *et al*., 2001). ACP and ALP are hydrolytic enzymes involved in transphophorylation which are important in the transportation of metabolites, metabolism of phospholipids, phosphoproteins, nucleotides and carbohydrates, and synthesis of proteins (Srivastava *et al*., 1995). Since AP is responsible for the splitting of esters at an alkaline pH and mediates membrane transport, the rise in its activity will enhance transport, cell growth and proliferation. Also high activity of the enzyme in the liver can activate phosphorylase enzymes promoting glycogen production thereby providing needed energy for coping with the exposure stress from toluene toxicity (Heath, 1991). Increased activity of ALP in the organs following exposure to toluene infers an increase production of the enzyme to combat the effect of toluene (Yakubu *et al*., 2005). Severe acidosis was reportedly responsible for the inhibition of ALP activity in intoxicated liver of *Sarotherodon mossambicus* exposed to sevin which was an adaptation for anaerobic breakdown of glycogen to meet energy demand (Shaikila *et al.,* 1993). Conversely, an alkaline environment in the liver with high activity of ALP may have enhanced aerobic breakdown of glycogen to meet the energy demands of the fish. Another consequence of ALP overproduction is the adverse effect on the facilitation of the transfer of metabolites across cell membranes. Increased activity in the organs coupled with a decrease in the plasma indicates that the integrity of the organs has not been compromised there is absence of tissue damage (Pari and Amali, 2005; Adedeji, 2010). The results from this study indicated that toluene did not affect the activities of the enzymes in the plasma, suggesting the integrity of the organs of *C. gariepinus* were intact, but in the organs and muscle tissues it enhanced their activities. Hence, the assessment of these enzymes in the organs and muscle tissues could be used as good biomarkers of toluene toxicosis.

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